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REVIEW

Communication Of Cancer Cells And Lymphatic Vessels In Cancer: Focus On Bladder Cancer

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Abstract: Bladder cancer is one of the most commonly diagnosed cancers worldwide and causes the highest lifetime treatment costs per patient. Bladder cancer is most likely to metastasize through lymphatic ducts, and once the lymph nodes are involved, the prognosis is poorly and finitely improved by current modalities. The underlying metastatic mechanism for bladder cancer is thus becoming a research focus to date. To identify relevant published data, an online search of the PubMed/Medline archives was performed to locate original articles and review articles regarding lymphangiogenesis and lymphatic metastasis in urinary bladder cancer (UBC), and was limited to articles in English published between 1998 and 2018. A further search of the clinical trials.gov search engine was conducted to identify both trials with results available and those with results not yet available. Herein, we summarized the unique mechanisms and biomarkers involved in the malignant progression of bladder cancer as well as their emerging roles in therapeutics, and that current data suggests that lymphangiogenesis and lymph node invasion are important prognostic factors for UBC. The growing knowledge about their roles in bladder cancers provides the basis for novel therapeutic strategies. In addition, more basic and clinical research needs to be conducted in order to identify further accurate predictive molecules and relevant mechanisms.

Keywords: bladder cancer, lymphangiogenesis, lymphatic metastasis, biomarkers, tumor progression, treatment

Introduction

UBC is the second most frequent malignant tumor of the urinary system, with the estimated 76,900 new cases, approximately 15,900 death in America during the year of 2016.[1,2] Smoking is just an established risk factor for UBC.[3] For histopathology, transitional cell carcinomas take account for nearly 90 percent of bladder cancers, the rest of UBC contains adenocarcinomas, squamous cell carcinomas, and undifferentiated bladder carcinomas.[4] UBC frequently show the peculiarity of progression, referring to two closely related processes of invasion and metastasis, and which could serve as the remarkable common cause involved with cancer-related deaths.[5] Concerning the natural process, bladder cancer is generally divided into two distinct groups, such as non-muscle invasive bladder cancer (NMIBC), also referred to as superficial, and muscle-invasive bladder cancer (MIBC). Among the superficial tumors, which often recurs and invades the muscle after the transurethral initial resection, up to 50–70% recurrence[6] and 10–20% progressing to MIBC,[7] respectively. MIBC may display as the high risk of metastatic malignant tumors and subsequently cause death, with the 5-year survival rate remarkably declining from 90% in NMIBC[8] to 60% in comparison with MIBC.[9] Even worse,

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approximately a quarter of patients suffering MIBC undergo radical cystectomy(refers to the removal of the bladder together with the prostate and seminal vesicles in this context), whereafter, unfortunately, show lymph node metastases later and die during the first five years after primary confirmed diagnosis.[10,11]

Tumor metastasis, including blood and lymphatic metastasis, is topmost of the dismal aspects of tumor progression. Besides, it is largely supposed that bladder cancer is most likely to disseminate from in-suit to distant organs through lymph ducts. Indeed, the detection of metastases within the sentinel lymph nodes is an essential prognostic factor for patient survival and select whether adjuvant therapies or not.[12] Once the lymph nodes are in invasion(Ln+) in the UBC, the poor prognosis will be defined in the future follow-ups,[13] contributing to about 90 percent of cancer-specific death.[14] Ln+ involves the new formation of lymphatic ducts in intratumoral and peritumoral regions at first, and next delivering of cancer cells to lymphatic vasculatures and spreading them to lymph nodes (LNs), finally inducing the settlement and proliferation of in LNs.[15,16]

It is anticipated that lymphangiogenesis plays vital roles in physiological (e.g., menstrual and hair cycle, ovarian follicle maturation, corpus luteum formation, and uterine implantation) and pathological (i.e., inflammation, wound healing and cancer.) processes. To date, the roles of lymphangiogenesis become a research hotspot in the aspect of unveiling mechanism of metastasis and exploring novel therapeutic strategies for individuals with urothelial carcinoma. It has been verified that lymphangiogenesis is an indispensable element for lymph nodes metastasis. Despite the increasing improvement in surgical techniques and adjuvant chemotherapy and immunotherapy, there are still poor treatment response and prognosis.[17] In recent, the treatment options for advanced UBC mainly depends on conventional clinicopathological characteristics, such as tumor grade and stage information, though providing important prognostic information in UBC, they are of limited use in the prediction of cancer recurrence, progression, treatment response, and survival,[18] partially due to the shortcomings of staging and grading subjectivity that can lead to highly observational error.[19] Fortunately, Ln+ is an earlier event when occurs the progression of MIBC with significantly predictive values, and the lymphatic vessels in or in proximity to tumors could serve as the primary conduits for the spread of cancer cells.[20] Moreover, thus, to unravel the lymphangiogenesis and lymph node invasion in UBC cries out for the treatment and surveillance in the future.

To date, the majority of research on lymphatics at the primary tumor has focused on the capacity of lymphatic vessels to facilitate the entry and transport of tumor cells; the influence of lymphatic location (intratumoral versus peritumoral); and the enlargement or collapse of the lymphatics during metastasis,[21,22] and the potential predictable roles for tumor grade and stage. This review highlights the current knowledge about the anatomical structure of the lymphatic system and underlying cellular mechanisms of lymphangiogenesis, discusses the fatal molecules and defining signals that control these processes, as the promising pools of anti-lymphangiogenic targets. The implications of these findings for the advancement of novel diagnostics and therapeutics, and future cancer research, are also discussed.

Lymphatic System And Its Role In Urothelial Carcinoma

Despite the lymphatic system initially described in the 17 centuries, it is generally regarded as the "forgotten" the second angiology.[23] In general, lymphatic conduits can fall into three separate types: initial blind-ended lymphatics, pre-collecting and collecting vessels.[23,24] Next, we discuss several well-characterized mediators involved the lymphatics development, and their interaction between lymphatics and tumor progression, especially for bladder cancer.

There are several well-known regulators that can modulate mammalian lymphatic system development, such as Prospero homeobox transcription factor-1 (PROX-1),[25] SRY-related HMG-box(SOX)-18,[26] and the vascular endothelial growth factor-C (VEGF-C)/VEGF receptor-3 (VEGFR-3) axis,[27] reader could extensively and comprehensively overview the mediator of the formation of the lymphatic system in several published reviews.[28–30] Enabling signal for lymphatic endothelial cells (LECs) differentiation is deemed to be regulated by mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling, and subsequently initiate SOX-18 to facilitate the upregulation of PROX-1,[31,32] which immediately leads to the PROX-1 expression to trigger the differentiation of LECs in turns, as characterized by the well-characterized lymphangiogenic genes expression including VEGFR-3, podoplanin, integrin a9, and chemokine (C-C motif) ligand 21(CCL21),[33,34] and also directs endothelial cells toward a lymphatic fate in vivo.[35] Further, the maintenance of LEC identity is reversible and dependent on PROX-1

expression,[36] which regulates VEGFR3 expression.[37] During the processes, the transcription factor musculoaponeurotic fibrosarcoma oncogene homolog B(MAFB) shows a potency in the maintenance of the LEC phenotype by overexpressing SOX-18 and PROX-1, while conversely, DLL4/Notch signaling downregulates the COUP transcription factor-2 (COUPTF-2)(also known as nuclear receptor subfamily 2 group F member 2; NR2F-2) and PROX-1 expression, maintaining venous endothelium cells characteristics. VEGF-C is an established lymphatic-associated growth factor.[38] VEGF-C crosstalks with its initial receptor, VEGFR-3, which activates the MAPK/ERK and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB/AKT) signaling pathways, triggering LECs survival, migration, and proliferation.[39] Contrarily, MAPK signaling is thought to be negatively mediated by the transcription factors fork head box protein C1 and C2 (FOXC1/FOXC2).[40]

It is starting as small initial blind-ended lymphatics that favor the absorption of interstitial fluid and cell in tissues.[41,42] The initial lymphatic capillaries, surrounded by intermittent basement membrane with no vascular smooth muscle cells or pericytes, are non-contracting vessels characterized by a single layer of LECs and abluminal membrane of LECs connected to surrounding interstitial elastic fibers by the short anchoring filaments,[42–44] and then flow into pre-collecting vessels and collecting vessels, further downstream, the collecting lymphatic vessels drain to the thoracic duct or the right lymphatic trunk, hereafter enter into the circulation systems[23,24,45,46] (Figure 1). In general, the lymphatic vasculature, functioning unidirectionally, is a drainage network that starts in the interstitial tissue and ends mainly in draining into the venous circulation, which serves as three of cardinal functions, such as maintaining interstitial fluid homeostasis,[47] immune surveillance and absorption of fat in the gastrointestinal tract.[23] The lymphatic system involves the local progression and invasion in urothelial carcinoma, Recent reports pay attention to the role of lymphangiogenesis and lymphatic vessel density (LVD) and indicate that both them could serve as a potential prognostic marker and as a mechanism of metastatic dissemination in those patients with UCB.[48,49] Intratumoral

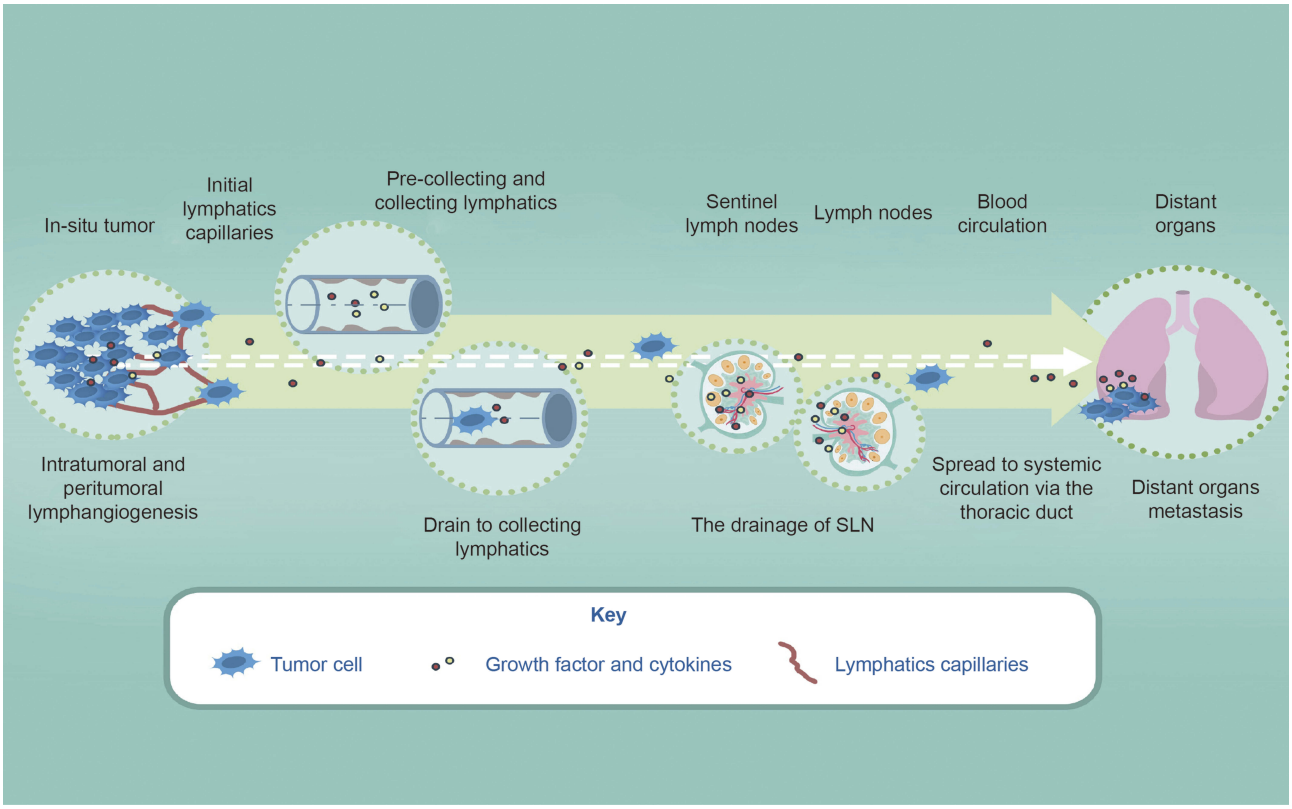


Figure 1 A simplified schematic of lymphatic metastasis in bladder cancer. Tumor cells in the tumor microenvironment produce factors (growth factors or cytokines) that induce sprouting growth of local initial lymphatic vessels. These factors, together with metastatic cells and immune cells, are transported via pre-collecting and collecting lymphatics to tumor-draining LNs. Tumor cells and other factors may then access systemic circulation through the thoracic duct, leading to spread to distant organs. Lymphangiogenesis in distant organs occurs as part of premetastatic niche formation and continues after the arrival of metastatic cells, potentially promoting further metastasis.
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lymphatics (ITLs) were quite small, irregular or flattened, and occasionally filled within tumor cells in the lumen, while peritumoral lymphatics(PTLs) were relatively disorganized and enlarged, and strictly located at the tumor periphery.[50] Morphological investigations have suggested that ITLs are newly proliferating, while PTLs are likely to become pre-existing lymphatic vessels. One study involved human breast cancer, indicated that LN metastasis could sufficiently proceed through the pre-existing lymphatic vessels, implying that lymphangiogenesis may not necessarily be involved at the tumor periphery regardless of ITL vessel density,[51] another investigation also indicated that ITLs is unnecessary for metastasis to LNs in urothelial carcinoma and prostate cancer.[52] Additionally, functional studies using assays for micro-lymphangiography and interstitial fluid pressure have indicated that ITLs may be nonfunctional. Meanwhile, multiple investigations in experimental models and human specimens have indicated that PTLs are more critical than ITLs for cancer metastasis cells.[53] However, a majority of studies also have reported an association between ITLs and PTLs and aggressive features of invasive UCB and upper tract urothelial carcinoma.[54,55] To conclude, it is unclear whether ITLs lack functions to date, ITLs can be detected in urothelial carcinomas conversely, despite the controversial role of ITLs lymphangiogenesis by neoplastic cells in regulating LNs metastasis yet, intratumoral lymphatics are high likelihood to sever as a risk factor for LNs metastasis and prognosis for patients with bladder cancer, thus targeting the intratumoral lymphatics is also imperative to diminish the spread of primary tumors as well as PTLs. More investigations need to explore and determine the precise underlying mechanism of how PTLs and ITLs to facilitate lymphatic metastasis in order to provide novel therapeutic strategies for individuals with urothelial carcinoma.

The Gene Expressing Profiling

It is generally understood the gene variation plays a vitally significant role in the emergence and development of carcinoma, associated with prognosis and cancer-specific survival as well. There are hundreds of thousands of gene mutation message to be distinguished on the basis of the fast development of sequencing technologies, however, the actual value of the gene expression signatures correlated with lymphangiogenesis and lymphatic metastasis in bladder cancer has not yet been elucidated, and we also do not make sure whether some of these gene alterations involved whether driver or passenger mutations. Nowadays, gene expression profiling for cancer has gained increasing attention owing to its ability to figure out a detailed and complete map to discover the molecular cancer subtypes at the transcriptome level, or based on the genetic and epigenetic alteration.

Several RNA-based expression data analysis assays indicate that gene differential expression in MIBC is associated with lymph node involvement, and to develop a list of gene expression models (GEM) predicts the pathological lymph node status in order to selecting patients for advanced neoadjuvant chemotherapy,[56–58] a research, however, indicated that predictive efficacy could not be validated merely based on a qRT-PCR platform.[59] Therefore, a novel gene screening method is advisable, which proposed 18-gene signatures highly predictive of lymphatic metastasis.[60] In recent, a study demonstrates a nomogram for preoperatively predicting LN metastasis in bladder cancer, which shows favorable discriminatory ability and may offer help for clinical decision-making.[61] A group fixes their concentration on the relationship between copy number variation and lymph node metastasis, consequently detect copy number gain at chr3p25 and chr11p11, approximately a set of 22 genes, which related to Ln+ and survival in bladder cancer.[62]

The Specific Biomarkers And Growth Factors For Lymphatic Systems

The exact molecular mechanism of lymphangiogenesis and lymph node metastasis was poorly influenced by the shortage of promising biomarkers that reasonably differ lymphatic vessels from blood vasculatures in the intratumoral and intertumoral areas. Luckily, recent studies focus their attention on lymphangiogenesis as well as the interests in LEC markers in UBC, various lymphatic-associated proteins have been detected such as PROX-1, VEGFR-3, SOX-18, NR2F2, neuropilin 2 (NRP-2), FOXC2, and others, some of which have important functions in the development of the lymphatic vasculature.[63,64] Nevertheless, only two of biomarkers have been widely implicated in neoplasm to identify newly-born lymphatics: lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE1) and podoplanin.[65] Multiple investigations give attention to the ITLs and PTLs, and their role in malignancy development and progression. Interestingly, Lymphovascular invasion (LVI)[66–69] and high LVDs[70–72] could be strongly prognostic factor for tumor aggressiveness and an indicator of occult metastases in several malignancies, including UCB as well,[13,73,74] while some retrospective analysis however controversially indicated LVDs is not associated with survival in various cancer in statistics (e.g.,

melanoma, breast cancer), thus, more research is needed in this part.

VEGF-C, VEGF-D, and their specific receptor, VEGFR-3 mark a profound milestone in the lymphatic commitment and metastasis, which are identified as guaranteeing prognostic indicators for malignant bladder cancer dissemination.[75] Additionally, several findings suggest that VEGF-C and VEGF-D are not just stimulate lymphangiogenesis but also may influence angiogenesis.[76] Indeed, some investigators reported that the roles of VEGF-C and VEGF-D associated with MVD to induce angiogenesis in cancer tissues.[77] However, other investigators have supported the hypothesis that VEGFC and VEGF-D do not have an angiogenic function.[78] Although numerous investigators speculated that the properties of VEGF-C and VEGF-D in lymphangiogenesis or angiogenesis might depend on the degree of their proteolytic processing,[76] clear explanations for such different functions of VEGF-C and VEGF-D were not provided by data from the present study. Current experimental and clinical studies mainly focus their attention on the roles of tumor-derived VEGF-C, VEGF-D, and their receptors for lymphangiogenesis and lymphatic metastasis, whereas tumor-associated stromal cells, especially stromal macrophages also could release VEGF-C and VEGF-D.[79] Therefore, further investigations need to clarify and understand VEGF-C/VEGF-D–VEGFR-3 axis as their potential in predictive biomarkers and novel therapeutic targets in patients with lymphatic metastasis. Neuropilin-2 was another factor with specific expression in lymphatic ducts,[80] which could regulate the VEGF signaling pathway due to the peculiarity of coreceptor.[81] The LYVE1, which positively applied to distinctively identify LVD, and thus promote the investigation of tumor-associated lymphangiogenesis in bladder cancer.[82]

Moreover, the transcription factor PROX-1 explicitly expressed in lymphatic vessel tissues but not blood vascular endothelium cell.[83,84] Furthermore, it has been reported that PROX-1 could upregulate the LEC-specific markers, whilst represses that of blood vascular endothelial cells (BECs) markers.[33,85] These in vitro and in vivo findings indicate that PROX-1 medicates the differentiation of LECs from embryonic BECs via functioning as a binary transcriptional switch, turning the BEC program off and the LEC program on. Since PROX-1 is recognized as a vital molecule to activate lymphatic development, molecular mechanisms how PROX-1 is induced during lymphatic development have been extensively investigated. For example, SOX-18, a member of the SOX family of transcription factors, directly binds to PROX-1 promoter and subsequently initiates its expression during the differentiation program of venous BECs to LECs.[26,86] Furthermore, PROX-1 requires the assistance of Ets family members for efficiently overexpressing the LEC-specific markers. To more specifically, several findings suggest that Ets-2 could stimulate expression of VEGFR-3, with the activation of its ligands-VEGF-C consistently, which suggests that cooperatively enhances PROX-1-induced lymphangiogenesis.[87]

Interestingly, the expression levels of VEGF-D, cyclooxygenase (COX)-2, and matrix metalloproteinase(MMP)-2 played vigorous influences in several processes, including malignant proliferation and lymphangiogenesis.[88] Also, podoplanin accurately express in lymphatic vessels, the upregulation of this kind of transmembrane glycoprotein could promote various human cancer to disseminate to distant organ based on epithelial-mesenchymal transition(EMT).[89] A commercially available antibody, named D2-40, correctly anchors human podoplanin[90] and has already been routinely monitored in UBC to investigate the lymphatics development.[91,92]

Lymphangiogenesis And Metastasis In Bladder Cancer

The VEGF-C/VEGF-D–VEGFR-3 Axis

Lymphatics proliferation is frequently observed in bladder cancer tissue, providing an extensive communicating area and facilitating bladder cancerous cell metastasis.[93] There is multistep processes involvement in the invasion and metastasis of cancer cells, including tumor cells permeate into adjacent lymphatic channels, transport tumorous cells through the lymphatic systems and plant into the distant tissues.[94] Lymphangiogenesis and lymphatic remodeling can induce cancer lymphatic metastasis by stimulating neoplastic cell invading into lymphatic vessels. However, the exact mechanism of lymphangiogenesis is unclear.[16,95,96] The VEGF-C/VEGF-D-VEGFR-3 axis is seen as a major driver of tumor lymphangiogenesis,[63] whereas the roles of other pathways in this process are less well defined. VEGF-C/VEGF-D-VEGFR-3 pathway stimulating proliferation and migration of LECs is such as to play vital roles in lymphangiogenesis and metastasis for bladder cancer.[97,98] Despite tyrosine kinase receptors, all three VEGFRs (e.g., VEGFR-1, −2 and −3) are not distributed on ECs equally. Indeed, VEGFR-1 mainly express on BECs with the feeble expression on LECs, VEGFR-3 expression is largely

restricted to lymphatic endothelial cells contrarily, whilst VEGFR-2 could be identified both on BECs and LECs. In human bodies, five different genes encode VEGF family members such as VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PlGF), respectively. According to their functions, it could be coarsely classified into two main groups, such as hematogenous factors (e.g., VEGF-A, VEGF-B, and PlGF) or lymphangiogenic factors (e.g., VEGF-C and VEGF-D).[99,100] To more precisely, lymphangiogenesis can be supported by VEGFR-3 and its homologous ligands including VEGF-C and VEGF-D. Moreover, the VEGFR-3 co-receptor NRP-2 regulates the signaling pathways that are undertaken in response to VEGF-C and VEGF-D.[101,102] These modes of initiating signals are somewhat analogous to the molecular modulation of angiogenesis by VEGF-A signaling via VEGFR-2 and NRP-1[65,103] (Figure 2). Proteolytic processing of VEGF-C has proved to increase its receptor affinity and biological activity.[104] Recent studies have implicated that two-star molecules that are required for the development of lymphatic vessels, such as disintegrin and metalloproteinase with thrombospondin motifs 3 (ADAMTS3) protease and collagen and calcium binding EGF domains 1 (CCBE1). Some research demonstrated that an ADAMTS3-CCBE1 complex could independently form, and such complexity is required to convert VEGF-C, but not VEGF-D, into an active ligand of with no reply on VEGFR3.[105] To be more specific, ADAMTS3 catalyzes the proteolytic processing of VEGF-C, removing the N-terminal propeptide and releasing the fully active, mature VEGF-C. In vivo, ADAMTS3 could efficiently activate VEGF-C signaling in cooperation with CCBE1. CCBE1 enhances the VEGF-C activation by two different mechanisms: it increases the processivity of the ADAMTS3 enzyme,[106] and it colocalizes ADAMTS3 and VEGF-C on extracellular matrix and cell surfaces to form the trimeric activation complex.[105] In addition, plasmin also shows its significant roles in activating the VEGF-C and VEGF-D to induce the new formation of lymphatic

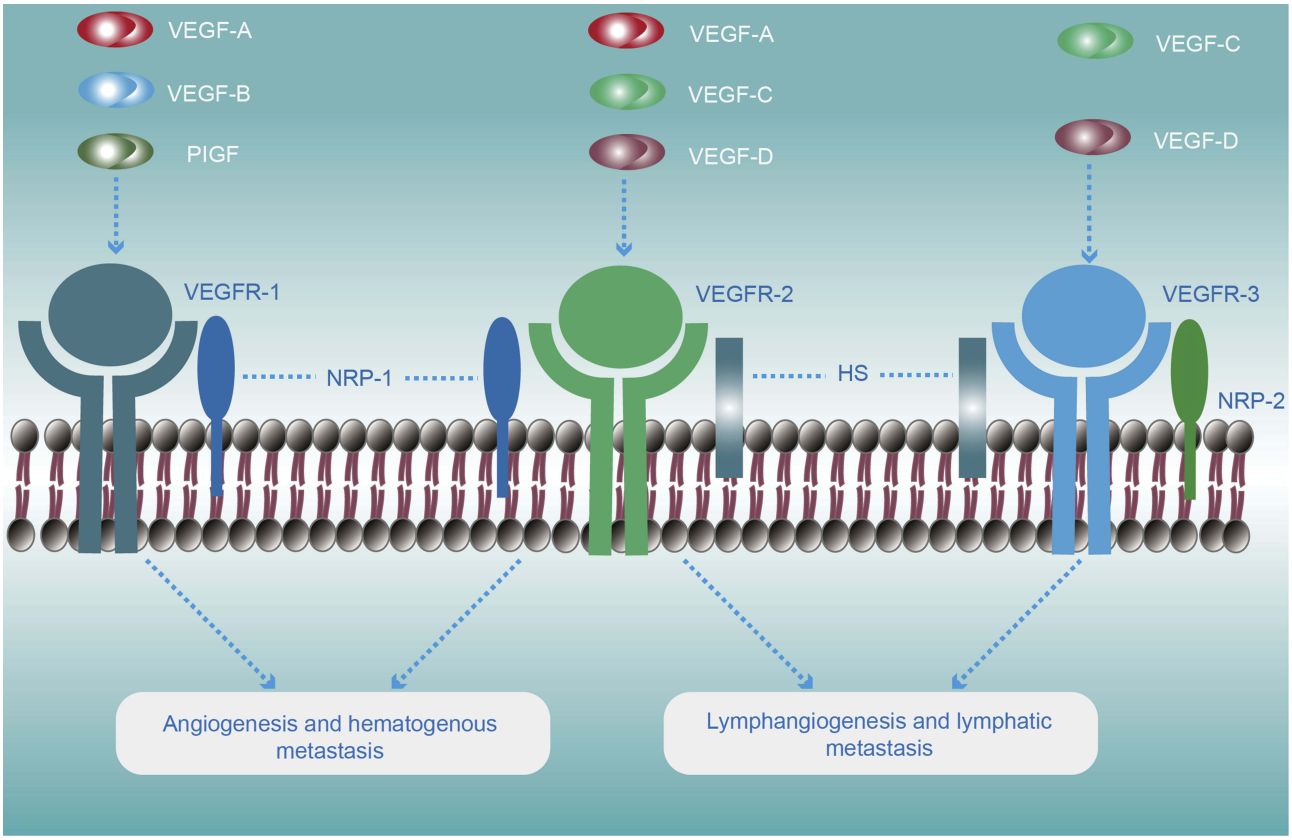


Figure 2 Schematic illustration of VEGFR structures and their specific ligands. VEGFRs are depicted as ligand-bound activated dimers. The VEGF-ligand family includes VEGFA, VEGFB, VEGFC, VEGFD, and the placenta growth factor (PlGF), which binds VEGFRs in a specific manner. VEGFB binds selectively to VEGFR-1. In contrast, VEGFA can activate VEGFR-2 and VEGFR-1, while VEGFC and VEGFD could anchor both VEGFR2 and VEGFR3 pathways. The VEGFRs co-receptors are indicated as NRP-1 and NRP-2 (neuropilin-1 and neuropilin-2, respectively) and HS (heparan sulfate; the main biological functions are listed below the respective receptors. In general, the VEGF-C/VEGF-D/VEGFR-3 pathway plays a fatal role in the lymphangiogenesis and lymphatic metastasis in bladder cancer, though VEGF-C/VEGF-D/VEGFR-2 pathway may participate to some extent.

vessels.[107] After plasmin and ADAMTS3, recent research identified several novel proteases including kallikrein-related peptidase 3 (KLK3) and cathepsin D with potential in activating of VEGF-C as well as VEGF-D to trigger the lymphangiogenesis and subsequently provide new perspectives to facilitate the development of inhibitors of cancer metastasis.[108]

The expression level of VEGF-C and VEGF-D is linked to LN+ and higher LVD, showing its potential function both in LN+ and lymphangiogenesis.[109] VEGF-C and VEGF-D motivate tumor cells to enter the circulatory system through enhancing tumor interstitial fluid pressure as a result of accelerating vascular leakage and lymph flow, as well increasing quantity, diameter and the proliferation rates of the LVD.[110,111] When the axis is enabled, the lymphangiogenesis can be detected within and peripheral tumors,[112] while the opposite outcome could be identified in some research while blocking the signaling pathway.[113]

The Ang2/Tie-2 Pathway

The angiopoietin system mainly includes Ang1, Ang2, and Ang4 and their receptors Tie-1 and Tie-2, and such a system has prominent impacts on endothelial cells both from the hemic and lymphatic system. Ang2, associated with tumor progression in bladder cancer,[114] is most noteworthy amongst the ligands in bladder cancer concerning the lymphatic system following that in experimental tumor models.[115] Moreover, Ang2 can contribute to the anatomical integrity of lymphatic systems during the embryogenesis.[116]

The HGF/c-MET Pathway

Hepatocyte growth factor (HGF) shows its functions in regulating resistance to anti-angiogenic therapies via changing tumor microenvironment,[117] and its receptor c-Met can be isolated from the cultured lymphatic endothelial cells in vitro, and upregulated when the occurrence of inflammation and cancer in vivo,[118] an indirect mechanism has also been described, mainly via activation of the VEGF-C/VEGF-D-VEGFR-3 axis.[119]

The Shh Signaling Pathway

In recent, the advent of the concept of cancer stem cell (CSC) has served as a novel concept for the development and progression of various tumor types, which immediately and indirectly participate in lymphangiogenesis.[120] The sonic hedgehog (Shh) pathway involves the new formation of lymphatic vessels in many tumors. Some studies demonstrate that Shh signaling activates the tumor metastasis and lymphangiogenesis through waking up the AKT, EMT, and the MMP-9 pathway.[121] When it now comes to UBC, Shh signaling also promotes oncogenesis and tumorigenicity,[122] but its role in tumor progression is undefined.[123] Some show that MIBC arises exclusively from Shh-expressing stem cells in the basal urothelium, and affirm that Shh expression is invariably lost as long as the progression to MIBC,[124–126] contrarily, others argue the constitutive activation of Shh signaling.[127] Cross-talks between Shh and TGF-beta may participate in the development and progression of bladder cancer as the consequence of manifesting EMT and bladder cancer stemness.[127–129] Furthermore, there are several studies unravel the correlation between the CSC and Shh signaling for the tumorigenesis in bldder.[130,131] A retrospective study shows that Shh pathway components are associated with lymphatic metastasis and poor clinical outcomes in bladder cancer.[132] We thus hypothesize that is worth to study Shh signaling pathway to figure out underlying therapeutic values for UBC in the future.

The CCL21/CCR7 Signal Pathway

Within most normal tissues, CCL21 mainly originates from the lymphatic system and binds to C-C chemokine receptor 7(CCR7) selectively expressing in activated DCs in normal conditions, thereby recruiting DCs toward the lymphatic systems to perform immune responses.[133] A study suggests the lymphatic flow plays a crucial role in upregulating the expression level of CCL21 of the endothelium in vitro.[134] However, various tumor cells have been verified that can positively label CCR7 by activating the MEK/ERK1/2 signaling pathway instead of the PI3K/AKT pathway.[135] CCR7 expression is in significant accordance with several clinicopathological parameters, including lymph node status, tumor stage, tumor grade, and overall survival in BC patients.[136] Both VEGF axis and TGF-β1 pathway could promote $CCR7^+$ tumor cells migrate towards LECs via upregulated CCL21.[137–139] In all, CCL21 is served as a probable carcinogenic factor due to its role in reversing the host immune response from immunogenic toward tolerogenic for $CCR7^+$ cancer cells.[140]

Others

There are several kinds of RNA molecules with no protein-coding capacity, including long non-coding RNAs (lncRNAs) and microRNAs (miRNAs). The diversity of lncRNAs shows vital roles in the development and progression of human cancers,[141] such as HOTAIR, SChLAP1. Moreover,

BLACAT1 are correlated with lymphatic metastasis.[142–144] Additional factors, including LINC01186 and lncRNA-ATB, participate in metastasis by inducing EMT.[145,146] A recent study indicates that lncRNAs markedly upregulated in LN-metastatic bladder cancer.[147] Many types of cancer are associated with aberrantly expressing of miRNAs,[148] such as miR-128 downregulation promotes lymphangiogenesis and metastasis of UBC by the upregulation of VEGF-C.[149] In addition to these that we have discussed above, multiple other growth factors and their receptors such as platelet-derived growth factor(PDGF)/PDGFR, fibroblast growth factor(FGF)-2/FGFR, epidermal growth factor(EGF)/EGFR and insulin-like growth factor(IGF)/IGFR involve with lymphangiogenesis under various circumstances, indirectly or directly.[64,65,150–153]

Therapeutics Aimed At Lymphangiogenesis And Lymphatic Metastasis

Nowadays, the first-line treatment for malignant MIBC is radical cystectomy with extensive pelvic lymphadenectomy, whereas a quarter of those patients undergoing radical cystectomy for MIBC show Ln+.[154] Nowadays, Ln+ is one of the most important predictors for the outcomes in individuals with bladder cancer. Cisplatin-based regimens have already regarded as the standard first-line chemotherapy since the late 1980s, including the use of including MVAC (methotrexate, vinblastine, adriamycin, and cisplatin) and GC (gemcitabine and cisplatin), and the survival of patients with metastatic urothelial carcinoma has yet remained rather poor.[6] Many studies demonstrate that the inhibition of the lymphangiogenic signaling pathway might restrict metastasis to lymph nodes and potentially to distant organs in various tumors, including urothelial carcinoma as well. Indeed, targeting tumor-associated lymphangiogenesis could be a feasible molecular target for patients with bladder cancer. The most studied biological pathway is VEGF-C/VEGF-D-VEGFR-3 axis, and such signaling is the important biological signaling pathway for lymphangiogenesis and lymphatic metastasis in tumors. As a consequence, inhibition of VEGF-C/VEGF-D/VEGFR-3 recognizes a potential way to restrain tumor progression.[155–157] Currently, the antibody of VEGF-C and its receptor antibody- VEGFR-3, such as VGX-100[158] and IMC-3C5,[159] respectively, have entered clinical testing for the treatment of cancer. In addition, blocking the VEGFR-2 also shows potential in diminishing the lymphatic metastasis.[160] Assorted other small-molecule antibodies and protein kinase inhibitors, under clinical trials and preclinical stages,[39,161–164] that target VEGF-C/VEGF-D-VEGFR-3 are shown in details in Table 1. Another signaling axis, such as the HGF-c-MET[165–168] and Ang2-Tie-2[169–171] pathway, may serve also as targets to inhibit lymphangiogenesis by specific antibodies (Table 1). Several antibodies and antibody formats such as Sorafenib,[172] Pazopanib,[173] Sunitinib,[174,175] Axitinib,[176] Regorafenib,[177] Vatalanib[178] have been extensively applied for cancer treatment in the clinic, could decline peritumoral LVD and lower the incidence of lymph node metastasis via inhabitation of various signaling pathways. Other potentially useful reagents, including neutralizing mAbs to NRP2[179] and COX enzymes,[180,181] are also in undergoing investigation, with showing potential therapeutic targets. However, it remains not clear as to when such drugs should be ideally offered to tumor patients to restrain carcinoma spreading. It is warranted to develop novel technology for patient selection pre-treatment, or prognosis surveillance after gave such anti-lymphangiogenic therapy. Immuno-positron emission tomography (I-PET) with lymphatic-specific antibodies, a new imaging approaches, may eke out from such assignable issues in the clinic.[182]

Cancer immunotherapy encompasses all the methods sought to facilitate the identification and eradication of cancerous cells by the aid of the immune system. Indeed, immunotherapies (e.g., BCG, CTLA-4, and PD-1/PD-L1) have revolutionized the treatment paradigm in urothelial carcinoma of the bladder, especially for the patients eventually develop resistance to this standard first-line treatments.[183,184] Booming evidence highlight that, aside from transport and trafficking functions, tumor-associated lymphatics further represent a conceivable role in shaping antitumor immunity. In particular, tumor-associated lymphatic vessels are initially required for the recruitment of immunocyte and the initiation of the adaptive immune response. Indeed, numerous investigations suggest that neoplasm drainage, dendritic cells trafficking, and subsequent activation of anti-tumor specific T cell responses is remarkably disturbed in transgenic mice with impaired or lacking local lymphatic vessels.[185,186] Antigens transport by dendritic cells via lymphatic vessels toward draining LNs is indispensable for the activation of anti-tumor adaptive immune responses, and thus indicate that develop LNs- or LVs-targeting approaches, including indirect targeting of draining LNs by the usage of drug delivery systems with optimal lymphatic retention and uptake properties, and the direct injection of immunomodulatory agents into cutaneous LNs or lymphatics, are potential therapeutics to impair malignancies progression and

Table 1 Overview Of Potential Drugs Which Target Lymphangiogenic Pathways

Target	Drug Name	Description	Status	NCT Or Ref Number
VEGF-C	VGX-100	VEGF-C blocking antibody	Phase I ongoing	NCT01514123
VEGF-D	VD1	Monoclonal antibodies	Preclinical	161, 162
	cVE199	Monoclonal antibodies	Preclinical	163
VEGF-C and VEGF-D	VGX-300	Soluble VEGFR3 construct	Preclinical	39
VEGF-C, VEGF-D, and VEGF-A	sVEGFR2	Soluble VEGFR2 construct	Preclinical	164
VEGFR-3	IMC-3C5/hF4-3C5	VEGFR-3 blocking monoclonal antibody	Phase I completed	NCT01288989
	Sorafenib	Small-molecule PKI	Approved for clinical applicant	172
	Pazopanib	Small-molecule PKI	Approved for clinical applicant	173
	Sunitinib	Small-molecule PKI	Approved for clinical applicant	174, 175
	Axitinib	Small-molecule PKI	Approved for clinical applicant	176
VEGFR-3 and TIE2	Regorafenib	Small-molecule PKI	Approved for clinical applicant	177
	CEP-11981	Small-molecule PKI	Phase I completed	NCT00875264
c-MET	AMG337	Small molecule c-MET inhibitor	Phase I completed Phase 2 terminated	NCT01253707 NCT02016534
	AMG 208	Small molecule c-MET inhibitor	Phase I completed Phase 2 terminated	NCT00813384 NCT02420587
	Crizotinib	Small molecule c-MET, ROS1and ALK	Phase 2 ongoing	NCT02034981
	PF-04217903	c-MET/HGFR tyrosine kinase inhibitors	Phase1 completed	NCT00706355
	Capmatinib (INC280)	Small molecule c-MET inhibitor	Phase I completed	NCT02626234
	Tepotinib (MSC2156119J)	Small molecule c-MET inhibitor	Phase I completed	NCT01832506
	Foretinib (GSK1363089 and XL 880)	Small molecule c-MET/HGFR inhibitors	Phase 2 completed	NCT00726323
	Tivantinib (ARQ 197)	Small molecule c-MET inhibitor	Phase 2 ongoing	NCT01892527
	ARGX 111	antibody blocking c-MET	Phase I completed	NCT02055066
	EMD 1204831	c-Met kinase Inhibitor	Phase I terminated	NCT01110083
	ABT-70 (ABBV-399)	anti-c-Met monoclonal antibody	Phase 2 ongoing	NCT03574753
	Volitinib (HMPL-504)	small molecule inhibitor of c-Met kinase	Phase I completed	NCT01773018
	Onartuzumab (MetMAb)	Monovalent, c-MET blocking antibody	Phase 3 completed	NCT01887886
	SAIT301	monoclonal antibody of c-MET	Phase I completed	NCT02296879

(*Continued*)

Table 1 (Continued).

Target	Drug Name	Description	Status	NCT Or Ref Number
EFG	YYB101	HGF neutralizing antibody	Phase 1 ongoing	NCT02499224
	Ficlatuzumab (AV-299)	HGF neutralizing antibody	Phase 1 ongoing	NCT03316599
	Rilotumumab (AMG 102)	HGF neutralizing antibody	Phase 3 terminated	NCT02137343
Ang1/Ang2	AMG 386 (Trebananib)	sequestering Ang1 and Ang2	Phase 3 terminated	NCT01281254
	CVX-060	Anti-angiogenic Covx-body Binding Ang2	Phase 1 completed	NCT00879684
	CVX-241	Ang2/VEGF neutralizing bisprecific CovX-body	Phase 1 completed	NCT01004822
	REGN910-3	Ang2 neutralizing antibody	Phase 1 completed	NCT02713204
	AMG780	Ang1/Ang2 neutralizing antibody	Phase 1 terminated	NCT01137552
TIE2	ARRY-614	p38/Tie2 inhibitor	Phase 1 completed	NCT01496495
	Regorafenib (BAY 73–4506)	Tie2 inhibitor	Phase 3 ongoing	NCT02773524
	DCC-2036	Tie2 inhibitor	Phase 1 completed	NCT00827138
	CEP-11981 (ESK981)	VEGFR/TIE2 tyrosine kinase inhibitor	Phase 1 ongoing	NCT03456804
	AMG-386	Neutralizing peptibody	Phase 2 completed	NCT01290263
NRP2	Anti-NRP2B	Monoclonal antibody	Preclinical	179
COX2	NSAIDs	Small molecules	Approved for use as analgesics and as anti-inflammatory agents	180, 181

Abbreviations: VEGF, vascular endothelial growth factor; PKI, protein kinase inhibitor; c-Met, tyrosine-protein kinase Met; HGFR, hepatocyte growth factor receptor; EFG, epidermal growth factor; Ang: angiotensin; TIE2, tyrosine kinase with immunoglobulin-like and EGF-like domains; NRP2, neuropilin 2; COX2, cyclooxygenase; NSAID: non-steroidal anti-inflammatory drug.

metastasis, which that are not discussed in there, readers can review in detail in several published review articles for these topics.[187–189] However, lymphatic endothelial cells, the initial components of lymphatic vessels, undergo active modifications that facilitate metastatic dissemination and can have direct interactions with immune cells (e.g., DCs and T cells) to negatively induce immunoregulation. To more specific, direct LEC-T cell interactions and antigen presentation to dendritic cells can induce the dysfunctional CD8+ T cell activation or the CD4+ T cell apoptosis,[187] or lymphatic endothelial cells produce several biological factors(e.g., IDO, MHCs, and NO) that indirectly aid in the maintenance of regulatory T cell populations and impairment of DCs maturation and T cell activation.[190–192] Thus, anchoring the LECs to manipulate immune responses open opportunities for therapeutic targeting in the cancer treatment paradigm. Additional research will need to determine how to selectively target LEC immunosuppressive functions in cancers, which could, combined with immunotherapeutic methods, facilitate the evolvement of a "cold" into "hot" immunogenic TME and potentiate anti-tumor T cell responses. The current clinical trial researches targeting at lymphatic metastasis involving cancers, and the above studies demonstrate that biological processes of lymphangiogenesis and anti-lymphangiogenesis involve multiple pathways. When one signaling pathway is inhibited, others may make compensation for its absence. Therefore, future anti-lymphatic treatment therapy may be complex involving inhibitors of diverse pathways.

Discussions

Over the past decades, tumor-associated lymphangiogenesis has been thought to be a new target to eliminate metastatic malignancy. To date, the development of specific anti-lymphangiogenic drugs has reached the stage of clinical trials. Nevertheless, much remains to be done: Primarily, the molecular mechanisms that govern the entry of tumorous cells into remodeled or newly-born lymphatic vessels-be their ITLs or PTLs-have not been comprehensively characterized; Second, it is unclear whether remodeling of lymphatics and/or the tumor-associated lymphangiogenesis are crucially required for the carcinoma dissemination to LNs or distant organs; Third, the degree to which LN metastases directly contribute to distant organ metastasis needs to be defined, as do the mechanisms through which this may occur; In addition, as mention above part, LVs play a dual role in tumor immunity; therefore, LV might display positive and/or negative effects on tumor immunity depending on the stage of tumor progression and on the immunological settings (immune evasion/immune subversion or immunotherapy). It is thus urgent to decipher precisely the roles for LVs in tumor cell dissemination and anti-tumor T cell immunity; Furthermore, novel imaging methods and whole-genome functional screens with LECs are needed to monitor the efficiency of anti-lymphangiogenesis drugs accurately, and to provide precise treatment for those patients who can get more benefits from these treatments; Additionally, anti-lymphangiogenic therapy may enhance interstitial fluid pressure and hamper drug delivery to cancerous cells.[193] Consistently with tumor angiogenesis, variety of lymphangiogenic pathways and hundreds of regulatory molecules involve the new formation of lymphatic vessels within TMEs, meaning that single pathway targeting drugs might not be efficient in all cases. Thereby, investigations into the optimal scheduling of combination therapies are needed.

Conclusions

Increasing clinical studies show the evidence that lymphatic vessel invasion and lymph node metastasis depict poor prognosis indicators in UBC. As already discussed, the GEP platform could analyze the relationships between tens of thousands candidate genes and lymph node involvement at a given time with the help of Next-Generation Sequencing and Biotechnology; however, GEP technology is limited due to unable to detect the interactions or signaling crosstalk. More investigations are also required to identify the genes associated with poor prognosis profiles, which could protect the patient against suffering poor prognosis via aiding in rational clinical decision-making during the earlier status. Over the past few decades, the great efforts have been made in elucidating the cellular and molecular mechanisms underlying lymphatic metastasis, especially the discovery of lymphangiogenic growth factors and specific biomarkers of LECs and development of GEM, making profound breakthroughs to unravel the complexity of the lymphatic metastatic process. Currently, the VEGF-C/VEGF-D-VEGFR-3 pathway is correlated with the tumor-derived lymphatic vessels and metastasis in UBC, blocking such signaling pathway suppresses tumor lymphangiogenesis. Research into the lymphatic system is currently undergoing another revolution about the real-time imaging of lymphatic metastasis, for instance, to develop a new, noninvasive in vivo imaging techniques contribute to detect metastases to evaluate the scope of surgery based on the identification of tumor-induced stromal changes, which mainly relies on the specific biomarkers. Owing to its complexity, the existing molecular mechanisms remain yet controversial. In the future, therefore, large-scale basic research and a grand mass of clinical specimens are in importance for developing further accurate molecules. Novel antitumor drugs targeting the newly identified molecular markers or pathway can then be put into place to prevent bladder cancer metastasis at an earlier stage and provide better outcomes for bladder cancer patients.

Abbreviations

UBC, urinary bladder cancer; NMIBC, non-muscle invasive bladder cancer; MIBC, muscle-invasive bladder cancer; LNs, lymph nodes; LEC, lymphatic endothelial; GEM, Gene expression models; MMP-2, matrix metalloproteinase-2; LVD, lymphatic vessels density; VEGF, vascular endothelial growth factor; PROX-1, prospero-related homeobox-1; COX-2, cyclooxygenase-2; EMT, epithelial-mesenchymal transition; HGF, hepatocyte growth factor; CSC, cancer stem cell; Shh, sonic hedgehog; DCs, dendritic cells; CCR7, C-C chemokine receptor 7; CCL21, chemokine (C-C motif) ligand 21; lncRNAs, long noncoding RNAs; miRNAs, microRNAs. SOX-18, SRY-related HMG-box-18, MAFB, musculoaponeurotic fibrosarcoma oncogene homolog B; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; COUPTF-2, COUP transcription factor-2; NR2F-2, nuclear receptor subfamily 2 group F member 2; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; NRP-2, neuropilin 2.

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Author Contributions

All authors contributed towards data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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